

Alarm calling best predicts mating and reproductive success in ornamented male fowl, *Gallus gallus*

WILSON ET AL.: BEHAVIOUR PREDICTS PATERNITY IN FOWL

DAVID R. WILSON*, KAREN L. BAYLY*, XIMENA J. NELSON*, MICHAEL GILLINGS[‡], AND
CHRISTOPHER S. EVANS*

*Centre for the Integrative Study of Animal Behaviour, Macquarie University

[‡]Genes to Geoscience Research Centre, Department of Biological Sciences, Macquarie University

Correspondence: D. R. Wilson, Centre for the Integrative Study of Animal Behaviour, Macquarie University, Sydney, NSW 2109, Australia (email: david@galliform.bhs.mq.edu.au). K. L. Bayly, X. J. Nelson, & C. S. Evans are at the Centre for the Integrative Study of Animal Behaviour, Macquarie University, Sydney, NSW 2109, Australia. M. Gillings is at the Genes to Geoscience Research Centre, Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia.

Word Count: 5429 words, excluding title page, abstract, references

Studies of female mate choice in fowl typically invoke ornament size as the best predictor of male reproductive success. The strongest evidence comes from experiments in which a hen is presented with two unfamiliar and physically separated males that she can evaluate and mate with for up to 120 minutes. This design controls for prior experience and male-male competition, but deprives females of information available only from longer sampling periods and a more natural context. In the wild, fowl spend their lives in stable social groups. We observed birds under naturalistic conditions to evaluate the biological significance of ornament size and to explore other potential predictors of male mating and reproductive success. For each male, we measured morphology and several behaviours related to food, predators, dominance, and courtship. Using principal components analysis and multiple regression, we show that behaviour is the best predictor of male mating and reproductive success under natural conditions, and that the most salient behaviours are dominance and the rate of antipredator signalling. Dominance probably affects an individual's reproductive success by determining access to receptive females, but the mechanism responsible for the role of alarm calling is less clear. Costly alarm signals may advertise male quality, or they may reflect judicious risk-taking by males that have achieved mating success.

Keywords: alarm call, fowl, *Gallus gallus*, ornament, paternity, referential signal, sexual selection

The hallmark of sexual selection is that phenotypic traits predict assortative mating and differential reproductive success (Andersson 1994). Identifying such traits is thus an essential first step in determining whether sexual selection is operating in a given species. Subsequent experiments can then be designed to test for a causal effect on reproductive success.

Individuals assessing a prospective mate or opponent often consider multiple cues that reflect an underlying quality (e.g., genetic quality, resource provisioning ability, fighting ability; Hagelin 2002; Candolin 2003). If preferred cues are unavailable, or if there is insufficient time to assess them accurately, animals may be forced to use less-reliable secondary cues (Zuk et al. 1992; Sullivan 1994). It is therefore important that experiments designed to identify cues relevant to sexual selection consider the life history of the species in question, and provide assessors with the gamut of cues and the integration time available to them in a more natural context (Sullivan 1990). Species that characteristically encounter rivals or prospective mates only briefly may have to rely on static morphological cues or transient displays that can readily be assessed. In contrast, when encounters with conspecifics are repeated or prolonged, as in species that form stable social groups, individuals can also consider facultative traits that require greater assessment time. A particularly striking example of such a process is provided by the superb fairy wren, *Malurus cyaneus*, in which female choice is dependent upon the date on which males moult into their nuptial plumage months earlier (Mulder & Magrath 1994).

Characters of particular interest to females have often been identified by presenting them with a simultaneous choice between a pair of unfamiliar and physically separated males (Zuk et al. 1990a). Similarly, observing the outcomes of aggressive interactions between dyads of unfamiliar males is a useful approach for identifying consistent differences between winners and losers of male-male competition (Hagelin 2002). However, female choice and male-male competition can act contemporaneously. Under these conditions, individual reproductive success will likely reflect an interaction between the two mechanisms of sexual selection, as opposed to the independent effect of either one (Qvarnström & Forsgren 1998). The possibility of such interactions requires verification of the

importance of traits identified in tests of either female choice or male-male competition in a more natural context, in which both mechanisms can play a role (Moore & Moore 1999).

Fowl are a classic and ideal system for studies of both female choice and male-male competition (Darwin 1871). Males are covered with long, brilliant, plumage, and their resistance to parasites is reflected in the size and condition of fleshy red ornaments (Zuk et al. 1990b; Parker & Ligon 2003). They engage in a courtship display known as 'waltzing', provision females with food, and protect them from predators (Kruijt 1964; Pizzari 2003). Females mate with multiple males (Ligon & Zwartjes 1995a) and exert cryptic female choice following insemination (Pizzari & Birkhead 2000). During fights for territory and rank, males use sharp spurs as weapons (Andersson 1994), and then assert their dominance by crowing and by a visual display known as 'wingflapping' (Kruijt 1964). Other males often interfere with copulation and intense sperm competition follows insemination (Kratzer & Craig 1980; Froman et al. 2002).

The vocal behaviour of fowl has received less attention from a functional perspective. Males have a large vocal repertoire (Collias 1987), which includes crowing and at least three types of referential signal (Evans 1997). Crowing is energetically inexpensive (Horn et al. 1995) and advertises a male's social status (Leonard & Horn 1995). Dominant males approach the crows of other dominant individuals, while females and subordinate males do not respond to crowing by males of any status (Leonard & Horn 1995). The three referential signals specifically predict the presence of food (Evans & Evans 1999), aerial predators and terrestrial predators (Evans et al. 1993). Companions respond in functionally appropriate ways: searching for food (Evans & Evans 1999), crouching while looking upward as though to detect a hawk, or standing erect while scanning the horizon as though to detect a fox (Evans et al. 1993). Food and aerial alarm calls are not produced reflexively, but rather depend upon the presence of a suitable audience. The necessary characteristics vary: any conspecific is sufficient to induce aerial alarm calling (Karakashian et al. 1988), while only hens provide an adequate audience for food calling (Evans & Evans 1999). Ground alarm calls are produced by both sexes and do not require an audience (Evans 1997).

We surveyed the literature for direct correlations between the phenotype and mating success of male fowl. In several mate choice experiments devoid of male-male competition (Zuk et al. 1990a, b, c; Zuk et al. 1992; Ligon & Zwartjes 1995a, b; Zuk et al. 1995a; Chappell et al. 1997; Ligon et al. 1998), females were presented with two unfamiliar and separated males for up to two hours. Females consistently preferred the male with the larger ornament (reviewed in Parker & Ligon 2003), suggesting that non-random mating is a function of female preference for parasite-resistant males (Zuk et al. 1990b). Several other studies, which did not measure ornamentation, found that a male's dominance is positively related to his mating success, suggesting that male-male competition is also important (Guhl et al. 1945; Guhl & Warren 1946; Kratzer & Craig 1980; Cheng & Burns 1988; Johnsen et al. 2001; Pizzari 2001, 2003).

In the present study, we measured possible correlates of male mating success in fowl living under naturalistic conditions. In addition to dominance and ornamentation, we evaluated the role of courtship behaviour and of referential signals evoked by food and predators. Courtship and food provisioning have been inconsistently associated with male mating success in previous studies (e.g. Zuk et al. 1995b; Pizzari 2003), but these traits are facultative and females may require more time to assess them than is available in conventional choice tests (Sullivan 1990). Antipredator behaviour is positively associated with male dominance (Pizzari 2003), but its relation with male mating success has not hitherto been assessed. Finally, we tested whether the traits identified as predictors of male mating success also predicted male reproductive success, to establish whether these are subject to selection.

METHODS

General Methods

Subjects were 64 male and 66 female sexually mature (1-6 year-old) fowl (*Gallus gallus*) derived from flocks of golden Sebrights that had been breeding freely for several generations. This strain has not

been artificially selected for rapid growth or egg production. Although morphologically distinct from junglefowl, they possess very similar behavioural and vocal repertoires (Collias 1987; Zuk et al. 1990c). All individuals were assigned at random to one of 22 social groups. These were each composed of three males and three females - a size and age structure consistent with that described for free-ranging fowl (Collias et al. 1966).

Birds were observed under naturalistic conditions in large outdoor aviaries during the austral breeding seasons (August-March) of 1999/2000 (season 1: three groups, $N_{\text{males}} = 9$), 2000/2001 (season 2: four groups, $N_{\text{males}} = 12$), 2005/2006 (season 3: 11 groups, $N_{\text{males}} = 31$; two males used in season 3 had been in groups tested previously and so did not contribute data to the analysis) and 2006/2007 (season 4: four groups, $N_{\text{males}} = 12$). A maximum of two groups were tested sequentially in any given aviary in any given season. Birds not involved in testing were housed in an indoor colony (see Evans & Evans 1999 for details). All birds were fitted with numbered and coloured leg bands to facilitate individual identification.

Groups were formed by simultaneously releasing all six birds into one of several large (10 m x 20 m), outdoor aviaries. These each contained a coop fitted with a perch for roosting, *ad libitum* food and water, grass with patches of bare ground for dustbathing, and a gazebo structure affording shelter from the sun. Aviaries were constructed of 1-cm² nylon mesh (A&A Contract Services, Qld, Australia), which provided birds with an unobstructed view of their surroundings. Following their initial release, we monitored all birds for signs of stress (e.g. panting). Overt aggression usually lasted less than one minute, always less than three minutes, and usually terminated when one bird signalled subordinate status by turning away. Following group formation, birds were given at least one week to establish stable social structure, acclimate to the new surroundings and habituate to humans, prior to data collection.

Behavioural Observations

We used continuous recording of a focal animal (Altmann 1974). In seasons 1 and 2, each male was observed for one 20-minute session per day for 12-25 days (range: 240-500 minutes/male; $X \pm SE = 411$

± 17 minutes, $N_{\text{males}} = 21$). Data collection for individual birds alternated daily between the morning (2-3 hours after sunrise) and afternoon (2-3 hours before sunset), and the order of observation of the three males in a group was randomized. The observer (KLB) either sat or stood in the middle of the aviary and scored behaviour using a notebook and a stopwatch. Observation of a group ended for the season when two of the three hens became broody (and hence sexually unreceptive). The operational sex ratio within groups therefore became male-biased during the sampling period, and females reluctant to become broody were represented for a longer duration. Dynamic sex ratios and differential periods of female receptivity are both characteristic of wild populations (Collias et al. 1966), and should not affect the relations between male phenotype and either mating or reproductive success.

In seasons 3 and 4, we observed each group for one 40-minute session per day over a 12-day period, at approximately the same times each day (0705-1115 hours or 1620-1920 hours AET). During a group's daily session, two of the three males were observed simultaneously by one of two observers (XJN or DRW) assigned to them at random. Each male was observed on eight of the 12 days (selected at random), for a total of 320 minutes. Observers sat on either side of the coop, which was located in the centre of one end of the aviary, and scored behaviour using JWatcher™ software (version 1.0) on a Macintosh laptop computer. At least one hen in each group was laying eggs during the 12-day observation period, but no hen became broody until after data collection for her group was complete.

During each focal session, we recorded the number of individual crows and the number of bouts (defined by intervening silences not exceeding five seconds) of aerial alarm calls, ground alarm calls and food calls produced by each focal male. Occasionally, males produce food calls in response to inedible objects (Gyger & Marler 1988) and alarm calls in response to innocuous species (Gyger et al. 1987). We could not always identify the item eliciting a food calling bout, or the perceived threat to which a male alarm called, but we commonly observed a genuine food item or threat in the vicinity of a vocalizing male. In addition to vocalizations, we also scored bouts of courtship waltzing and wingflapping (Kruijt 1964). As waltzing can also be an aggressive display towards other males (Kruijt 1964), we considered only those bouts in which a female was within one metre of the focal male and no rival males were

within this radius. We scored male-male interactions involving a focal animal as a 'win' if the focal male displaced the other male and a 'loss' if he was displaced by him. Our criteria for displacements required that the two males were within one metre of each other, and that movement of one (defined by taking at least one step away) occurred within one second of movement by the other. All males within a group interacted at least once. Finally, we estimated each male's reproductive success by scoring all copulations, defined as the male grasping the back of a female's neck with his mandibles and mounting her with both feet.

Following data collection, we converted each male's total number of crows, ground alarm calls, aerial alarm calls, food calls, courtship waltzes, wingflaps, and copulations observed during all observation sessions into average rates/hour to facilitate comparisons across periods of unequal duration. As social status may affect a male's behaviour and mating success (Collias et al. 1966), we calculated a dominance score for each male using Kalinoski's (1975) Frequency Success Index (FSI), which is the most appropriate measure for this system (Bayly et al. 2006). FSI is calculated by subtracting an individual's losses from its wins, and dividing the difference by the total number of interactions in the group. The result for each male ranges between -1 (most subordinate) and +1 (most dominant).

Morphological Measurements

Immediately following each group's observation period, we captured each male, measured his body weight (accuracy: ± 10 g) using a Pesola™ spring scale and a cloth bag and took a digital photograph in right side profile (Canon EOS 300 digital camera; 6.5 megapixels resolution). For consistency with previous mate-choice studies, we measured the maximum length of the comb. We then measured the size of all head and facial ornaments from the digital images (Fig. 1a) using NIH ImageJ software (versions 1.62 and 1.33u), calibrated on a scale that had been placed beside each male's head. Specifically, we measured the total red surface area of the comb, wattle, ear lappet, and red facial skin (accuracy: ± 1 mm²), thereby estimating the size of the ornament in two of its three dimensions.

Comb and feather colour have also been shown to affect mate choice, although previous measurements of colour (Zuk et al. 1990b, c) have used subjective techniques such as the Munsell system, which has been criticized for its reliance upon a perceptual model of human vision (Bennett et al. 1994). During season 3 ($N_{\text{males}} = 31$), we used a USB2000 Miniature Fiber Optic Spectrometer (Ocean Optics, Inc., Dunedin, FL, USA) to measure the reflectance spectra of each male's comb and hackle feathers at four randomly selected locations on each structure (Fig. 1). Reflectance was measured relative to a white WS-1 diffuse reflectance standard (reflectivity: > 99%; wavelength range: 200-1100 nm) using a two-fibre probe. Illumination was provided by a MINI-D2T miniature deuterium tungsten light source (Ocean Optics, Inc; peak-to-peak stability: 0.3% from 200-850 nm). Measurements were taken using OOIBase32 spectrometer operating software at 0.37-nm increments between 350 and 700 nm, which corresponds to the complete spectral sensitivity of the fowl visual system (Prescott & Wathes 1999). Finally, we calculated a median reflectance spectrum from each male's four measurements for the comb and for the hackle feathers. We then standardized the reflectance spectra, such that for each structure the highest median reflectance value received a score of one and the lowest a score of zero.

Paternity Analysis

We conducted a paternity analysis in season 3 on a subset of seven groups (21 males and 21 females). A total of 97 eggs laid during the 12-day observation periods were collected and incubated at 38.3°C and 85% relative humidity. Embryonic development was stopped by chilling at 72 hours and all tissue was dissected and placed into 70% ethanol. At the end of each observation period, we used a 21-gauge needle to draw approximately 1 ml of blood from the brachial vein of every adult in the social group. All samples were stored at -20°C.

DNA was extracted from 42 adults and 71 embryos (26 eggs were not fertilized) using a proteinase K/salting out method (Sunnucks & Hales 1996). For all samples, microsatellite loci were amplified using approximately 50 ng of genomic DNA in 50- μ l reactions using the procedures outlined in Curley & Gillings (2004). Polymerase chain reactions (PCRs) contained 2 mM MgCl_2 , 200 μ M of each dNTP, 20

μg/ml RNaseA, 0.5 μM of each primer, and 0.15 units of Red Hot DNA polymerase (Advanced Biotechnologies) in the buffer supplied with the enzyme. Amplifications were made with a Hybaid Omne cycler and PCR conditions were 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 s, 48-62°C for 30 s (see Appendix A for details), and 72°C for 90 s, with a final extension at 72°C for 5 minutes. An aliquot of each PCR was electrophoresed on 2% agarose and stained with ethidium bromide to confirm amplification. PCRs were then diluted 1:10 in sterile water and analyzed on a 3130xl Genetic Analyzer (Applied Biosystems). Primer sets with nonoverlapping allele sizes and different fluorochrome labels were pooled for analysis to minimize time and costs. Allele sizes were measured using GeneMapper (version 4.0) software (Applied Biosystems) relative to internal LIZ™-labeled GeneMapper 500 size standards from Applied Biosystems.

Genotype matching was done manually using a first-principles approach because each group was a closed system in which the genotypes of all offspring and potential parents were known. We constructed a 3 x 3 matrix for each embryo, in which the columns and rows represented the genotypes of potential fathers and potential mothers, respectively. At each locus, the embryo's genotype was examined and all parental combinations that violated the Mendelian assumption that each parent had donated one randomly-selected allele to the embryo were eliminated from the matrix. Remaining cells in the matrix were assigned an equal probability, such that the sum of all remaining cells was one. Each adult's probability of parentage was equal to the sum of his or her respective row or column. An identified parent thus obtained a score of one and an excluded parent a score of zero. This procedure was repeated for every embryo in the group. Each male's probability of paternity was summed across all embryos within his group; this total provided an individual estimate of a male's cumulative reproductive success. Note that this score does not reflect inter-female variation in reproductive success (see Appendix B for details of both inter-male and inter-female variation), and hence should be used cautiously when considering the precise mechanisms of sexual selection that might underlie the observed variation in male reproductive success. Nevertheless, cumulative reproductive success is the most appropriate measure for our purpose because selection for traits that affect male reproductive success, whether it acts

through male-male competition or female choice, will be dependent on a male's overall fitness, as opposed to the number of females that contribute to it.

Statistical Analysis

We examined 10 potential predictors of male mating frequency ($N_{\text{males}} = 64$) and reproductive success ($N_{\text{males}} = 21$), including dominance (FSI), body weight, ornamentation (total red area of the ornaments and comb length), and the rates of crowing, ground alarm calling, aerial alarm calling, food calling, courtship waltzing, and wingflapping (see Table 1 for descriptive statistics). Each male contributed only one observation to each dataset to preserve independence (Machlis et al. 1985). Because independent variables were numerous and inter-correlated (see Table 2 for correlation matrix), we first performed a principal components analysis with varimax rotation to reduce the 10 independent variables in each dataset to three orthogonal factors (Table 3), thereby simplifying the datasets for exploration. We used multiple regression analysis to assess the statistical significance of each factor as a predictor of male mating and reproductive success.

Using the original data, we also tested the statistical significance ($\alpha = 0.05$) of the 10 independent variables using a multiple regression model and a forward stepwise selection procedure ($P \leq 0.05$ to add, $P \geq 0.10$ to remove). Residuals derived from a preliminary version of the model predicting mating success were not normally distributed, so we used a \log_{10} -transformation (one-sample Kolmogorov-Smirnov test of normality: $P > 0.05$ following transformation; Chatterjee et al. 2000). We also assessed the fit of each predictor variable by independently regressing it against each dependent variable and examining the residuals. Weight, when regressed against mating success, violated the assumption of normality, but was improved by a \log_{10} -transformation (one-sample Kolmogorov-Smirnov test of normality: $P > 0.05$ following transformation). Transformed variables were used in all analyses, including the principal components analysis.

We tested predictor variables for possible multicollinearity by examining variance inflation factors (VIF; Chatterjee et al. 2000). VIFs greater than ten indicate potential problems associated with multicollinearity (Chatterjee et al. 2000); our greatest VIF was 2.01 in the model predicting mating success (Table 4) and 5.30 in the model predicting reproductive success (Table 5). Our final models complied with all of the assumptions of linear regression (Chatterjee et al. 2000).

The reflectance properties of combs and feathers have not previously been tested for their effects on female mate choice in fowl. The potentially salient region(s) of the colour spectrum therefore remain unknown. For each character (comb and hackle feathers), we compared the reflectance spectra of the 10 males with the highest copulation rates to those of the 10 males with the lowest copulation rates in season 3. Interquartile ranges were plotted for each group of males and areas along the spectrum where these failed to overlap were considered to be statistically different from each other. A separate analysis of colour relative to paternity was unnecessary, because the assignment of males to groups was identical to that based upon mating frequency.

RESULTS

The factor analysis reduced the original 10 variables in each dataset to three orthogonal factors, which together accounted for 69.8% (mating success dataset, $N = 64$) and 79.2% (reproductive success dataset, $N = 21$) of the original variation. Factor loading scores presented in Table 3 show that, for both datasets, factor one best explained dominance, behaviours related to dominance (crows, wingflaps), and referential signalling (ground alarm calls, aerial alarm calls, food calls). Factor two best explained the traits related to sexual advertisement, including courtship waltzing, total red area of the ornaments, and comb length. Factor three best explained body weight. Only factors one and three explained a significant amount of the variation in mating (multiple regression analyses: $F_{3,60} = 19.93$, $P < 0.01$, $R^2_{\text{adjusted}} = 0.47$; factor 1: $t = 7.42$, $P < 0.01$; factor 2: $t = 0.10$, $P = 0.92$; factor 3: $t = -2.19$, $P = 0.03$) and reproductive success ($F_{3,17} = 6.29$, $P < 0.01$, $R^2_{\text{adjusted}} = 0.44$; factor 1: $t = 3.21$, $P < 0.01$; factor 2: $t = -1.94$, $P = 0.07$; factor 3: $t = -2.20$, $P = 0.04$) (Fig. 2).

The multiple linear regression model and forward stepwise selection procedure accounted for a significant amount of the variation observed in both mating frequency ($F_{3,60} = 24.93$, $P < 0.01$, $R^2_{\text{adjusted}} = 0.53$) and reproductive success ($F_{2,18} = 7.83$, $P < 0.01$, $R^2_{\text{adjusted}} = 0.41$). Mating success was predicted by aerial alarm calls, dominance, and (inversely) by body weight (Table 4), while reproductive success was predicted by ground alarm calls and (inversely) by the total red area of the ornaments (Table 5). Although several of the included variables were intercorrelated (Table 2), their high partial correlation coefficients (Tables 4 & 5) show that they account for considerable variation in mating and reproductive success, even after controlling for the effects of the other predictors. With the exception of aerial alarm calls (test for equality of slopes (Zar 1996): $P < 0.05$), regressions of mating and reproductive success on any given trait were remarkably concordant (Fig. 2) and statistically indistinguishable (all $P > 0.1$), suggesting that mating and reproductive success related similarly to the predictor variables. Mating frequency did not, however, predict reproductive success directly (simple linear regression analysis: $t_{19} = 0.721$, $P = 0.48$).

Finally, we could detect no differences in the colour of either combs or hackle feathers between males that were highly successful in mating and those that were not (Figs 1b & c). For each structure, the inter-quartile bands of the two groups overlapped considerably across the entire spectrum to which fowl are sensitive (350-700 nm), despite significant differences in their rates of copulation (lowest-mating males, $\bar{X} \pm \text{SE}$: 0.02 ± 0.01 copulations/hour; highest-mating males: 0.38 ± 0.04 copulations/hour; unpaired t -test: $t_{18} = 9.47$, $P < 0.01$). These reflectance characteristics hence provide no sensory basis for either female mate choice or opponent assessment.

DISCUSSION

Mating and reproductive success were directly related to dominance, as would be expected given the pervasive importance of social status in this system (Schjelderup-Ebbe 1935). Surprisingly, production of referential signals was also important. The best predictor of both mating and reproductive success was

the rate at which males produced antipredator alarm calls. This is the first such demonstration in any species. The relations between alarm calling and mating / reproductive success persisted even after controlling for the effects of dominance. They were also quite specific to potentially costly alarm signals (Marler 1955; Alatalo & Helle 1990; Wood et al. 2000). Food calling, crowing, and wingflapping predicted mating and reproductive success, but only to the extent that these attributes were associated with social status (see also Pizzari 2003). Overall, behavioural aspects of male phenotype accounted for almost half of the total observed variation in mating and reproductive success. Well-documented post-copulatory mechanisms (Pizzari & Birkhead 2000) likely account for much of the remainder.

In marked contrast to previous experimental mate choice studies (reviewed in Parker & Ligon 2003), we found no significant relation between mating frequency and male ornament size, while the relation between ornament size and reproductive success was negative. In these previous tests, females had been given between 30 and 120 minutes to observe and mate with either of two males. Males could not physically interact with each other, so females had no information about relative dominance (Zuk et al. 1990a). Females were also initially unfamiliar with the males. They hence had insufficient time to assess traits that require integration over hours or days, such as individual differences in rates of facultative signalling (Sullivan 1990). Female preference for male ornamentation might therefore be a secondary assessment strategy used by females when primary cues, such as dominance and signalling behaviour (Sullivan 1990; Zuk et al. 1992), are unavailable. In nature, females sometimes encounter unfamiliar males from other groups (Collias et al. 1966). During such transient encounters they may rely on the size and condition of a male's ornamentation, which provides an instantaneous 'snapshot' of his underlying quality, resistance to parasites, and prospect as an extragroup mate (Sullivan 1990; Zuk et al. 1990b).

Male morphology may have been unimportant in our study because female preferences for it were obscured by the effects of male-male competition (e.g. Petersson et al. 1999), a factor excluded in experimental choice tests. Alternatively, our inability to detect a role for ornaments in particular may have been due to morphological differences between our birds (Fig. 1a) and red junglefowl, which have a more pronounced 'row' comb. Note, however, that variation in comb length among the males used in our

social groups (coefficient of variation: 14.0%) was substantially greater than that in previous studies that have revealed a role for ornament size (coefficient of variation among 48 males in 1987: 9.2%; Zuk et al. 1990c). Hens hence failed to express a preference, even though they had ample perceptual information with which to do so. Further experimentation will be necessary to better understand the relative importance of cue availability, assessment time, conflict between intersexual and intrasexual selection, and strain morphology in this system.

Mating frequency failed to predict reproductive success, a finding which probably reflects well-documented post-copulatory mechanisms (see also Bilcik & Estevez 2005). Fowl are highly promiscuous and exhibit cryptic female choice, female sperm storage, differential sperm allocation, and sperm competition (Brillard 1993; Ligon & Zwartjes 1995a; Pizzari & Birkhead 2000; Froman et al. 2002; Pizzari et al. 2003). All of these mechanisms can act to de-couple male mating from reproductive success, which might explain why ornamentation was unrelated to mating frequency (Table 4), but inversely related to reproductive success (Table 5) in the present study. Using natural social groups, Bilcik & Estevez (2005) showed that a male's comb size did not predict how often females would solicit matings from him, but was positively related to his probability of mating forcibly (Bilcik & Estevez 2005). It is thus possible that females may respond to forced copulations by large-combed males by ejecting their sperm hence reducing their paternity.

The evolution of alarm calling is a classic problem in behavioural biology. Signallers risk predation by warning conspecifics of impending danger (Alatalo & Helle 1990), yet obtain no obvious benefits in return. Kin selection (Maynard Smith 1965) has often been invoked as a potential explanation. In Belding's ground squirrels (*Spermophilus beldingi*), for example, females are significantly more likely to alarm call if their offspring are within view (Sherman 1977). Similar phenomena have been described in round-tailed ground squirrels (*S. tereticaudus*), Sonoma chipmunks (*Eutamias sonomae*), black-tailed and Gunnison's prairie dogs (*Cynomys ludovicianus*; *C. gunnisoni*), yellow-bellied marmots (*Marmota flaviventris*), and Siberian jays (*Perisoreus infaustus*), suggesting that female alarm calling functions to

warn descendent, and potentially non-descendent, kin (Dunford 1977; Smith 1978; Hoogland 1983; Hoogland 1996; Blumstein et al. 1997; Griesser & Ekman 2004).

Males of these species also produce alarm calls, but the reason for this is less clear (Dunford 1977; Sherman 1977; Smith 1978; Hoogland 1983; Hoogland 1996; Blumstein et al. 1997; Griesser & Ekman 2004). Males are not philopatric, so warning non-descendent kin is unlikely. In addition, multiple mating by females diminishes a male's certainty of paternity and, consequently, the direct benefits he might obtain from warning her young (Hare et al. 2004). Males could selectively warn offspring, but this would require either that they recognize their own young using a phenotypic marker, or that they remain resident in their offspring's natal territory so that a spatiotemporal rule has the same functional effect. As previous studies (Dunford 1977; Sherman 1977; Smith 1978; Hoogland 1983; Hoogland 1996; Blumstein et al. 1997; Griesser & Ekman 2004) have not established paternity, it remains unknown whether the presence of descendent kin affects male alarm calling effort. More generally, a link between male mating success and alarm calling effort has not previously been reported for any species.

The strong predictive relation between male alarm calling and reproductive success apparent in our study offers a new insight into the evolution of this signal. Male alarm calling provides females with protection from predators (Kruijt 1964). In addition, the risk associated with alarm calling (Marler 1955; Alatalo & Helle 1990; Wood et al. 2000) may advertise the male's ability to shun predators, since only individuals best able to evade attack should be able to increase their conspicuousness with impunity. Alarm calling in fowl is testosterone-dependent (Gyger et al. 1988), and high levels of testosterone are known to impose significant physiological costs by compromising immune function (Zuk et al. 1995a), so high rates of alarm calling may also reflect superior health and resistance to parasites. Our findings are thus consistent with the idea that male alarm calling is a sexually-selected trait that has evolved via female choice (Zahavi 1975; Hamilton & Zuk 1982).

It is also possible that alarm calling reflects judicious investment in mates and prospective offspring by males that have achieved recent mating success. Male dunnocks (*Prunella modularis*), for example,

434 adjust their chick-feeding effort according to the proportion of matings obtained (Davies et al. 1992).
435 Similarly, male willow tits (*Parus montanus*) increase their rate of alarm calling when their mate is
436 within sight (Hogstad 1995). In many avian species, mating is associated with elevated testosterone titre
437 (Moore 1982). This provides a possible androgen mechanism by which the production of alarm calls and
438 mating / reproductive success might be linked. The male investment and female choice models outlined
439 here are not, of course, mutually exclusive. Further experiments are needed to elucidate the causal
440 relationship(s) between alarm calling and mating / reproductive success in fowl.

441
442 In conclusion, fowl are one of the best studied examples of sexual selection, yet the context in which
443 this work has been done has often been artificial (Sullivan 1990). Under naturalistic social conditions,
444 and with a more extended sampling period, male reproductive success was not positively related to
445 ornamentation. Instead, our results show that referential signalling and dominance both predict male
446 mating and reproductive success, and that the best predictor among those examined is a male's rate of
447 antipredator alarm signalling.

448

Acknowledgments

This research adhered to the Animal Behavior Society's *Guidelines for the Use of Animals in Research* and was conducted in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (NHMRC, 1997). All procedures were approved under Macquarie University AEC protocols 99002 and 2006/025. We thank N. Lambert, W. McTegg, and R. Miller for bird care, P. Taylor and A. Taylor for assistance with the statistical analysis, M. Holley and P. Worden for assistance with the paternity analysis, and A. Cockburn, J. Hare, and several anonymous reviewers for comments on previous drafts of the manuscript. This research was supported by a Sigma Xi Grant-in-Aid-of Research and Newton Society Sponsorship to KLB and by an Australian Research Council Discovery-Project Grant to CSE.

References

- Alatalo, R. V. & Helle, P.** 1990. Alarm calling by individual willow tits, *Parus montanus*. *Animal Behaviour*, **40**, 437-442. doi: 10.1016/S0003-3472(05)80523-8.
- Altmann, J.** 1974. Observational study of behavior: sampling methods. *Behaviour*, **49**, 227-267.
- Andersson, M.** 1994. *Sexual selection*. New Jersey: Princeton University Press.
- Bayly, K. L., Evans, C. S. & Taylor, A.** 2006. Measuring social structure: a comparison of eight dominance indices. *Behavioural Processes*, **73**, 1-12. doi: 10.1016/j.beproc.2006.01.011.
- Bennett, A. T. D., Cuthill, I. C. & Norris, K. J.** 1994. Sexual selection and the mismeasure of color. *American Naturalist*, **144**, 848-860. doi: 10.1086/285711.
- Bilcik, B. & Estevez, I.** 2005. Impact of male-male competition and morphological traits on mating strategies and reproductive success in broiler breeders. *Applied Animal Behaviour Science*, **92**, 307-323.
- Blumstein, D. T., Steinmetz, J., Armitage, K. B. & Daniel, J. C.** 1997. Alarm calling in yellow-bellied marmots: II. The importance of direct fitness. *Animal Behaviour*, **53**, 173-184. doi: 10.1006/anbe.1996.0286.

477 **Brillard, J. P.** 1993. Sperm storage and transport following natural mating and artificial insemination.
 478 *Poultry Science*, **72**, 923-928.

479 **Candolin, U.** 2003. The use of multiple cues in mate choice. *Biological Reviews*, **78**, 575-595.

480 **Chappell, M. A., Zuk, M., Johnsen, T. S. & Kwan, T. H.** 1997. Mate choice and aerobic capacity in
 481 red junglefowl. *Behaviour*, **134**, 511-529.

482 **Chatterjee, S., Hadi, A. S. & Price, B.** 2000. *Regression analysis by example*. 3rd edn. New York: John
 483 Wiley & Sons, Inc.

484 **Cheng, K. M. & Burns, J. T.** 1988. Dominance relationship and mating behavior of domestic cocks-a
 485 model to study mate-guarding and sperm competition in birds. *Condor*, **90**, 697-704. doi:
 486 10.2307/1368360.

487 **Collias, N. E.** 1987. The vocal repertoire of the red junglefowl: a spectrographic classification and the
 488 code of communication. *Condor*, **89**, 510-524. doi: 10.2307/1368641.

489 **Collias, N. E., Collias, E. C., Hunsaker, D. & Minning, L.** 1966. Locality fixation, mobility and social
 490 organization within an unconfined population of red jungle fowl. *Animal Behaviour*, **14**, 550-559. doi:
 491 10.1016/S0003-3472(66)80059-3.

492 **Curley, B. G. & Gillings, M. R.** 2004. Isolation of highly polymorphic microsatellite loci from the
 493 temperate damselfish *Parma microlepis*. *Molecular Ecology Notes*, **4**, 551-553. doi:
 494 10.1111/j.1471-8286.2004.00729.x.

495 **Darwin, C. R.** 1871. *The Descent of Man, and Selection in Relation to Sex*. Princeton: Princeton
 496 University Press.

497 **Davies, N. B., Hatchwell, B. J., Robson, T. & Burke, T.** 1992. Paternity and parental effort in
 498 dunnocks *Prunella modularis*: how good are male chick-feeding rules? *Animal Behaviour*, **43**,
 499 729-745. doi: 10.1016/S0003-3472(05)80197-6.

500 **Dunford, C.** 1977. Kin selection for ground squirrel alarm calls. *American Naturalist*, **111**, 782-785. doi:
 501 10.1086/283206.

502 **Evans, C. S.** 1997. Referential signals. *Perspectives in Ethology*, **12**, 99-143.

503 **Evans, C. S. & Evans, L.** 1999. Chicken food calls are functionally referential. *Animal Behaviour*, **58**,
 504 307-319. doi: 10.1006/anbe.1999.1143.

505 **Evans, C. S., Evans, L. & Marler, P.** 1993. On the meaning of alarm calls: functional reference in an
 506 avian vocal system. *Animal Behaviour*, **46**, 23-38. doi: 10.1006/anbe.1993.1158.

507 **Froman, D. P., Pizzari, T., Feltmann, A. J., Castillo-Juarez, H. & Birkhead, T. R.** 2002. Sperm
 508 mobility: mechanisms of fertilizing efficiency, genetic variation and phenotypic relationship with
 509 male status in the domestic fowl, *Gallus gallus domesticus*. *Proceedings of the Royal Society of*
 510 *London, Series B*, **269**, 607-612. doi: 10.1098/rspb.2001.1925.

511 **Griesser, M. & Ekman, J.** 2004. Nepotistic alarm calling in the Siberian jay, *Perisoreus infaustus*.
 512 *Animal Behaviour*, **67**, 933-939. doi: 10.1016/j.anbehav.2003.09.005.

513 **Guhl, A. M. & Warren, D. C.** 1946. Number of offspring sired by cockerels related to social dominance
 514 in chickens. *Poultry Science*, **25**, 460-472.

515 **Guhl A. M., Collias, N. E. & Allee, W. C.** 1945. Mating behavior and the social hierarchy in small
 516 flocks of white leghorns. *Physiological Zoology*, **18**, 365-390.

517 **Gyger, M., Karakashian, S. J., Dufty, A. M. Jr. & Marler, P.** 1988. Alarm signals in birds: the role of
 518 testosterone. *Hormones and Behaviour*, **22**, 305-314.

519 **Gyger, M. & Marler, P.** 1988. Food calling in the domestic fowl, *Gallus gallus*: The role of external
 520 referents and deception. *Animal Behaviour*, **36**, 358-365.

521 **Gyger, M., Marler, P. & Pickert, R.** 1987. Semantics of an avian alarm call system: The male domestic
 522 fowl, *Gallus domesticus*. *Behaviour*, **102**, 15-40.

523 **Hagelin, J. C.** 2002. The kinds of traits involved in male-male competition: a comparison of plumage,
 524 behavior, and body size in quail. *Behavioral Ecology*, **13**, 32-41. doi: 10.1006/anbe.2000.1618.

525 **Hare, J. F., Todd, G. & Untereiner, W. A.** 2004. Multiple mating results in multiple paternity in
 526 Richardson's ground squirrels (*Spermophilus richardsonii*). *Canadian Field-Naturalist*, **118**, 90-94.

527 **Hamilton, W. D. & Zuk, M.** 1982. Heritable true fitness and bright birds: a role for parasites? *Science*,
 528 **218**, 384-387. doi: 10.1126/science.7123238.

529 **Hogstad, O.** 1995. Alarm calling by willow tits, *Parus montanus*, as mate investment. *Animal*
 530 *Behaviour*, **49**, 221-225. doi: 10.1016/0003-3472(95)80170-7.

531 **Hoogland, J. L.** 1983. Nepotism and alarm calling in the black-tailed prairie dog (*Cynomys*
 532 *ludovicianus*). *Animal Behaviour*, **31**, 472-479. doi: 10.1016/S0003-3472(83)80068-2.

533 **Hoogland, J. L.** 1996. Why do Gunnison's prairie dogs give anti-predator calls?
534 *Animal Behaviour*, **51**, 871-880. doi: 10.1006/anbe.1996.0091.

535 **Horn, A. G. & Leonard, M. L. & Weary, D. M.** 1995. Oxygen consumption during crowing by
536 roosters: talk is cheap. *Animal Behaviour*, **50**, 1171-1175.

537 **Johnsen, T. S., Zuk, M. & Fessler, E. A.** 2001. Social dominance, male behaviour and mating in
538 mixed-sex flocks of red jungle fowl. *Behaviour*, **138**, 1-18. doi: 10.1163/156853901750077754.

539 **Kalinoski, R.** 1975. Intra- and interspecific aggression in house finches and house sparrows. *Condor*, **77**,
540 375-384. doi: 10.2307/1366086.

541 **Karakashian, S. J., Gyger, M. & Marler, P.** 1988. Audience effect on alarm calling in chickens
542 (*Gallus gallus*). *Journal of Comparative Psychology*, **102**, 129-135. doi:
543 10.1037/0735-7036.102.2.129.

544 **Kratzer, D. D. & Craig, J. V.** 1980. Mating behavior of cockerels: effects of social status, group size
545 and group density. *Applied Animal Ethology*, **6**, 49-62. doi: 10.1016/0304-3762(80)90093-0.

546 **Kruijt, J. P.** 1964. Ontogeny of social behaviour in Burmese red junglefowl, (*Gallus gallus spadiceus*).
547 *Behaviour*, **Supplement 12**, 1-201.

548 **Leonard, M. L. & Horn, A. G.** 1995. Crowing in relation to social status in roosters. *Animal Behaviour*,
549 **49**, 1283-1290.

550 **Ligon, J. D. & Zwartjes, P. W.** 1995a. Female red junglefowl choose to mate with multiple males.
551 *Animal Behaviour*, **49**, 127-135. doi: 10.1016/0003-3472(95)80160-X.

552 **Ligon, J. D. & Zwartjes, P. W.** 1995b. Ornate plumage of male red junglefowl does not influence mate
553 choice by females. *Animal Behaviour*, **49**, 117-125. doi: 10.1016/0003-3472(95)80159-6.

554 **Ligon, J. D., Kimball, R. & Merola-Zwartjes, M.** 1998. Mate choice by female red junglefowl: the
555 issues of multiple ornaments and fluctuating asymmetry. *Animal Behaviour*, **55**, 41-50. doi:
556 10.1006/anbe.1997.0582.

557 **Machlis, L., Dodd, P. W. D. & Fentress, J. C.** 1985. The pooling fallacy: problems arising when
558 individuals contribute more than one observation to the data set. *Zeitschrift für Tierpsychologie*, **68**,
559 201-214.

560 **Marler, P.** 1955. Characteristics of some animal calls. *Nature*, **176**, 6-8. doi: 10.1038/176006a0.

561 **Maynard Smith, J.** 1965. The evolution of alarm calls. *American Naturalist*, **99**, 59-63.

562 **Moore, M. C.** 1982. Hormonal response of free-living male white-crowned sparrows to experimental
563 manipulation of female sexual behavior. *Hormones and Behaviour*, **16**, 323-329.

564 **Moore, A. J. & Moore, P. J.** 1999. Balancing sexual selection through opposing mate choice and male
565 competition. *Proceedings of the Royal Society of London, Series B*, **266**, 711-716. doi:
566 10.1098/rspb.1999.0694.

567 **Mulder, R. A. & Magrath, M. J. L.** 1994. Timing of prenuptial molt as a sexually selected indicator of
568 male quality in superb fairy-wrens (*Malurus cyaneus*). *Behavioral Ecology*, **5**, 393-400. doi:
569 10.1093/beheco/5.4.393.

570 **Parker, T. H. & Ligon, J. D.** 2003. Female mating preferences in red junglefowl: a meta-analysis.
571 *Ethology Ecology & Evolution*, **15**, 63-72.

572 **Petersson, E., Järvi, T., Olsén, H., Mayer, I. & Hedenskog, M.** 1999. Male-male competition and
573 female choice in brown trout. *Animal Behaviour*, **57**, 777-783.

574 **Pizzari, T.** 2001. Indirect partner choice through manipulation of male behaviour by female fowl, *Gallus*
575 *gallus domesticus*. *Proceedings of the Royal Society of London, Series B*, **268**, 181-186. doi:
576 10.1098/rspb.2000.1348.

577 **Pizzari, T.** 2003. Food, vigilance, and sperm: the role of male direct benefits in the evolution of female
578 preference in a polygamous bird. *Behavioral Ecology*, **14**, 593-601. doi: 10.1093/beheco/arg048.

579 **Pizzari, T. & Birkhead, T. R.** 2000. Female feral fowl eject sperm of subdominant males. *Nature*, **405**,
580 787-789. doi: 10.1038/35015558.

581 **Pizzari, T., Cornwallis, C. K., Løvlie, H., Jakobsson, S. & Birkhead, T. R.** 2003. Sophisticated sperm
582 allocation in male fowl. *Nature*, **426**, 70-74. doi: 10.1038/nature02004.

583 **Prescott, N. B. & Wathes, C. M.** 1999. Spectral sensitivity of the domestic fowl (*Gallus g. domesticus*).
584 *British Poultry Science*, **40**, 332-339.

585 **Qvarnström, A. & Forsgren, E.** 1998. Should females prefer dominant males? *Trends in Ecology &*
586 *Evolution*, **13**, 498-501. doi: 10.1016/S0169-5347(98)01513-4.

587 **Sherman, P. W.** 1977. Nepotism and the evolution of alarm calls. *Science*, **197**, 1246-1253. doi:
588 10.1126/science.197.4310.1246.

589 **Schjelderup-Ebbe, T.** 1935. Social behaviour of birds. In: A Handbook of Social Psychology (Ed. by C.
590 Murchison), pp. 947-972. New York: Russell and Russell.

591 **Smith, S. F.** 1978. Alarm calls, their origin and use in *Eutamias sonomae*. *Journal of Mammalogy*, **59**,
592 888-893. doi: 10.2307/1380172.

593 **Sullivan, M. S.** 1990. Assessing female choice for mates when the males' characters vary during the
594 sampling period. *Animal Behaviour*, **40**, 780-782. doi: 10.1016/S0003-3472(05)80709-2.

595 **Sullivan, M. S.** 1994. Mate choice as an information gathering process under time constraint:
596 implications for behaviour and signal design. *Animal Behaviour*, **47**, 141-151. doi:
597 10.1006/anbe.1994.1016.

598 **Sunnucks, P. & Hales, D. F.** 1996. Numerous transposed sequences of mitochondrial cytochrome
599 oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and*
600 *Evolution*, **13**, 510-524.

601 **Wood, S. R., Sanderson, K. J. & Evans, C. S.** 2000. Perception of terrestrial and aerial alarm calls by
602 honeyeaters and falcons. *Australian Journal of Zoology*, **48**, 127-134. doi: 10.1071/ZO99020.

603 **Zahavi, A.** 1975. Mate selection-a selection for a handicap. *Journal of Theoretical Biology*, **53**, 205-214.
604 doi: 10.1016/0022-5193(75)90111-3.

605 **Zar, J.H.** 1996. *Biostatistical Analysis*. 3rd edn. New Jersey: Prentice-Hall, Inc.

606 **Zuk, M., Johnson, K., Thornhill, R. & Ligon, J. D.** 1990a. Mechanisms of female choice in red jungle
607 fowl. *Evolution*, **44**, 477-485. doi: 10.2307/2409430.

608 **Zuk, M., Thornhill, R. & Ligon, J. D.** 1990b. Parasites and mate choice in red jungle fowl. *American*
609 *Zoologist*, **30**, 235-244. doi: 10.1093/icb/30.2.235.

610 **Zuk, M., Thornhill, R., Ligon, J. D., Johnson, K., Austad, S., Ligon, S. H., Thornhill, N. W. &**
611 **Costin, C.** 1990c. The role of male ornaments and courtship behavior in female mate choice of red
612 jungle fowl. *American Naturalist*, **136**, 459-473. doi: 10.1086/285107.

613 **Zuk, M., Ligon, J. D. & Thornhill, R.** 1992. Effects of experimental manipulation of male secondary
614 sex characters on female mate preference in red jungle fowl. *Animal Behaviour*, **44**, 999-1006. doi:
615 10.1016/S0003-3472(05)80312-4.

- 616 **Zuk, M., Johnsen, T. S. & Maclarty, T.** 1995a. Endocrine-immune interactions, ornaments and mate
617 choice in red jungle fowl. *Proceedings of the Royal Society of London, Series B*, **260**, 205-210. doi:
618 10.1098/rspb.1995.0081.
- 619 **Zuk, M., Popma, S. L. & Johnsen, T. S.** 1995b. Male courtship displays, ornaments and female mate
620 choice in captive red jungle fowl. *Behaviour*, **132**, 821-836.

621 **Appendix A.** Microsatellite loci used to establish paternity in seven groups of fowl

622

623

624

625

626

627

628

629

630

631

632

633

634

635

636

Locus*	Primer sequences (5'-3') [†]	Repeat	T _a °C	Size range	<i>k</i>	<i>H_o</i>
ADL0176 (G01598)	F: tetTTGTGGATTCTGGTGGTAGC R: TTCTCCCGTAACACTCGTCA	(GT) ₁₂	48	180-200	4	0.07
ADL268 (G01688)	F: famCTCCACCCCTCTCAGAACTA R: CAACTTCCCATCTACCTACT	(GT) ₁₂	48	108-112	2	0.26
LEI0192 (Z83797)	F: famTGCCAGAGCTTCAGTCTGT R: GTCATTACTGTTATGTTTATTGC	(CTTT) ₁₂	58	254-266	2	0.58
LEI0221 (Z83791)	F: nedCCTTTATCCACTCTTCATGCAC R: TGCATAAATCCATGGGTAAGC	(CTTT) ₂₁	62	205-211	2	0.49
LEI0243 (Z94843)	F: petTTCAAATCTGTCACTGGAAAGG R: CAGGGTGCATGTGTATCATACC	(GAAA) ₂₆	62	189-205	4	0.48
LEI0258 (DQ239559)	F: famCACGCAGCAGAACTTGGTAAGG R: AGCTGTGCTCAGTCCTCAGTGC	((CTTT) ₂ CCTT) ₁₈	54	251-307	2	0.48

637

* Locus name and GenBank accession number

638

[†] Primers were 5' end labeled with the indicated fluorochrome

639

Shown are the locus name, primer sequences, repeat motif, annealing temperature (T_a °C), observed size range, number of observed alleles (*k*), and observed

640

heterozygosity (*H_o*). All adults (21 males, 21 females) and embryos (*N* = 71) were genotyped for all loci, except ADL268, where *N*_{embryos} = 58.

641 **Appendix B.** Individual reproductive success in seven groups of fowl

642	Group	Embryos	Male	Female 1	Female 2	Female 3
643						
644	1	2	1	0.17	0.00	0.00
645			2	0.17	0.17	1.17
646			3	0.17	0.17	0.00
647	2	14	1	0.33	0.00	1.67
648			2	0.00	2.00	8.33
649			3	0.33	0.67	0.67
650	3	4	1	0.00	0.14	0.14
651			2	0.00	0.81	0.81
652			3	0.81	0.64	0.64
653	4	9	1	0.00	0.93	0.93
654			2	0.42	1.02	1.27
655			3	1.08	1.08	2.27
656	5	21	1	2.92	0.47	2.22
657			2	4.67	0.42	4.67
658			3	2.92	0.47	2.22
659	6	9	1	0.00	0.00	2.50
660			2	1.50	0.00	1.00
661			3	3.50	0.00	0.50
662	7	12	1	0.25	0.17	1.17
663			2	0.25	2.42	6.92
664			3	0.00	0.17	0.67
665						

666 Males and females are arranged arbitrarily within their respective groups.

667 Values represent the estimated reproductive success for each parental combination. See text for details of

668 how estimates were derived.

Table 1. Descriptive statistics for 10 potential predictors of male mating frequency ($N = 64$) and reproductive success ($N = 21$)

Variable	Mean	SE	Minimum	Maximum	CV (%)
Dependent					
Matings	0.19	0.03	0.00	0.75	106.3
Paternity	3.38	0.68	0.17	10.33	92.5
Independent					
Crows	11.17	1.11	0.00	35.44	79.2
Ground alarm calls	1.73	0.34	0.00	16.36	155.3
Aerial alarm calls	3.97	0.40	0.19	14.32	79.6
Food calls	1.14	0.16	0.00	5.44	112.9
Courtship waltzes	1.79	0.21	0.00	8.25	94.7
Wingflaps	6.69	0.46	0.25	15.75	54.6
Dominance (FSI)	0.00	0.08	-0.89	1.00	62.1
Weight (kg)	1.10	0.02	0.76	1.48	14.1
Ornament area (cm ²)	27.23	0.60	17.39	40.22	17.6
Comb length (cm)	6.83	0.12	4.72	8.94	14.0

Note: SE: standard error; CV: coefficient of variation. The coefficient of variation for FSI was calculated using $FSI + 1$ to avoid division by zero. Data are presented prior to transformation. Matings, vocalizations, and visual displays are expressed as rates per hour.

690 **Table 2.** Correlations among 10 potential predictors of male mating success

691

692 Variable	Crow	Ground	Aerial	Food	Waltz	Wingflap	Dominance	Weight	Ornament	Comb
693		alarm	alarm	call					area	length
694 Crow	.	0.23	0.00*	0.01*	0.00*	0.00*	0.00*	0.62	0.15	0.07
695 Ground alarm	0.15	.	0.00*	0.00*	0.84	0.01*	0.00*	0.02*	0.04*	0.22
696 Aerial alarm	0.53*	0.55*	.	0.00*	0.01*	0.00*	0.00*	0.26	0.18	0.25
697 Food call	0.32*	0.53*	0.48*	.	0.99	0.01*	0.00*	0.20	0.26	0.23
698 Waltz	0.37*	0.03	0.35*	0.00	.	0.01*	0.27	0.02*	0.01*	0.01*
699 Wingflap	0.52*	0.32*	0.50*	0.34*	0.35*	.	0.00*	0.03*	0.02*	0.06
700 Dominance	0.55*	0.50*	0.61*	0.40*	0.14	0.62*	.	0.76	0.93	0.74
701 Weight	-0.06	-0.30*	-0.14	-0.16	0.30*	0.27*	-0.04	.	0.00*	0.00*
702 Ornament area	0.18	-0.25*	0.17	-0.14	0.34*	0.29*	-0.01	0.46*	.	0.00*
703 Comb length	0.23	-0.16	0.15	-0.15	0.33*	0.24	-0.04	0.36*	0.82*	.
704										

705 Note: Pairwise Pearson correlation coefficients are presented below the diagonal and *P*-values above the diagonal (2-tailed, *N* = 64, statistical significance

706 indicated by an asterisk ($\alpha = 0.05$)). Weight has been log₁₀-transformed. All vocalizations and visual displays are based upon rates per hour.

Table 3. Principal components analysis of male morphology and behaviour

Variable	<i>N</i> = 64 males			<i>N</i> = 21 males		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
Crow	0.64	0.42	-0.05	0.78	0.40	-0.18
Ground alarm	0.73	-0.24	-0.19	0.94	0.06	-0.05
Aerial alarm	0.82	0.29	-0.15	0.75	0.54	-0.07
Food call	0.70	-0.19	-0.07	0.52	-0.03	0.29
Waltz	0.26	0.55	0.31	0.40	0.70	-0.29
Wingflap	0.69	0.25	0.48	0.83	0.23	0.35
Dominance	0.83	-0.01	0.17	0.82	-0.09	0.15
Weight	-0.18	0.26	0.89	0.14	0.18	0.91
Ornament area	-0.08	0.88	0.23	0.03	0.94	0.23
Comb length	-0.07	0.90	0.08	-0.01	0.93	0.20
% variance	33.7	23.4	12.7	38.7	27.8	12.7

Note: Factor loading scores are presented for the complete dataset (*N* = 64 males) and for a subset of the data in which paternity is known (*N* = 21 males). Each factor is comprised of a linear combination of the 10 variables. The square of each factor loading score represents the proportion of variance in the relevant measure predicted by that factor. Weight was log₁₀-transformed in the *N* = 64 dataset. All vocalizations and visual displays are based upon rates per hour. Orthogonal rotation method: varimax

Table 4. Coefficients table for predictors of male mating success

Included:	<i>B</i>	SE	<i>Beta</i>	<i>t</i>	<i>P</i>	<i>r</i>	VIF
Intercept	0.81	0.29		2.77	0.01		
Aerial alarm	0.01	0.00	0.42	3.79	0.00	0.44	1.64
Dominance	0.04	0.01	0.34	3.13	0.00	0.38	1.61
Weight	-0.26	0.10	-0.23	-2.67	0.01	-0.33	1.03
Excluded:				<i>t</i>	<i>P</i>	<i>r</i>	VIF
Crow				-1.61	0.11	-0.20	1.57
Food call				1.27	0.21	0.08	1.35
Waltz				-0.69	0.49	0.16	1.36
Alarm				0.59	0.56	-0.09	1.67
Wingflap				0.26	0.80	0.03	2.01
Ornament area				-0.21	0.84	-0.03	1.42
Comb length				0.16	0.88	0.02	1.26

Note: *B*: Unstandardized coefficient; SE: standard error; *Beta*: Standardized coefficient; *r*: partial correlation coefficient; VIF = variance inflation factor. Variables were entered into a multiple regression model using a forward stepwise procedure ($P \leq 0.05$ to add, $P \geq 0.10$ to remove; $N = 64$). Mating success and weight were \log_{10} -transformed.

Table 5. Coefficients table for predictors of male reproductive success

Included:	<i>B</i>	SE	<i>Beta</i>	<i>t</i>	<i>P</i>	<i>r</i>	VIF
Intercept	8.97	2.96		3.03	0.01		
Ground alarm	2.27	0.75	0.52	3.04	0.01	0.58	1.00
Ornament area	0.00	0.00	-0.46	-2.64	0.02	-0.52	1.00
Excluded:				<i>t</i>	<i>P</i>	<i>r</i>	VIF
Weight				-1.60	0.13	0.19	1.15
Wingflap				1.27	0.22	0.18	3.14
Food call				-1.14	0.27	-0.27	1.31
Waltz				1.02	0.32	0.24	2.13
Comb length				0.87	0.40	0.29	5.30
Dominance				0.81	0.43	0.19	1.88
Crow				0.79	0.44	-0.36	2.90
Aerial alarm				0.74	0.47	0.21	4.12

Note: *B*: Unstandardized coefficient; SE: standard error; *Beta*: Standardized coefficient; *r*: partial correlation coefficient; VIF = variance inflation factor. Variables were entered into a multiple regression model using a forward stepwise procedure ($P \leq 0.05$ to add, $P \geq 0.10$ to remove; $N = 21$).

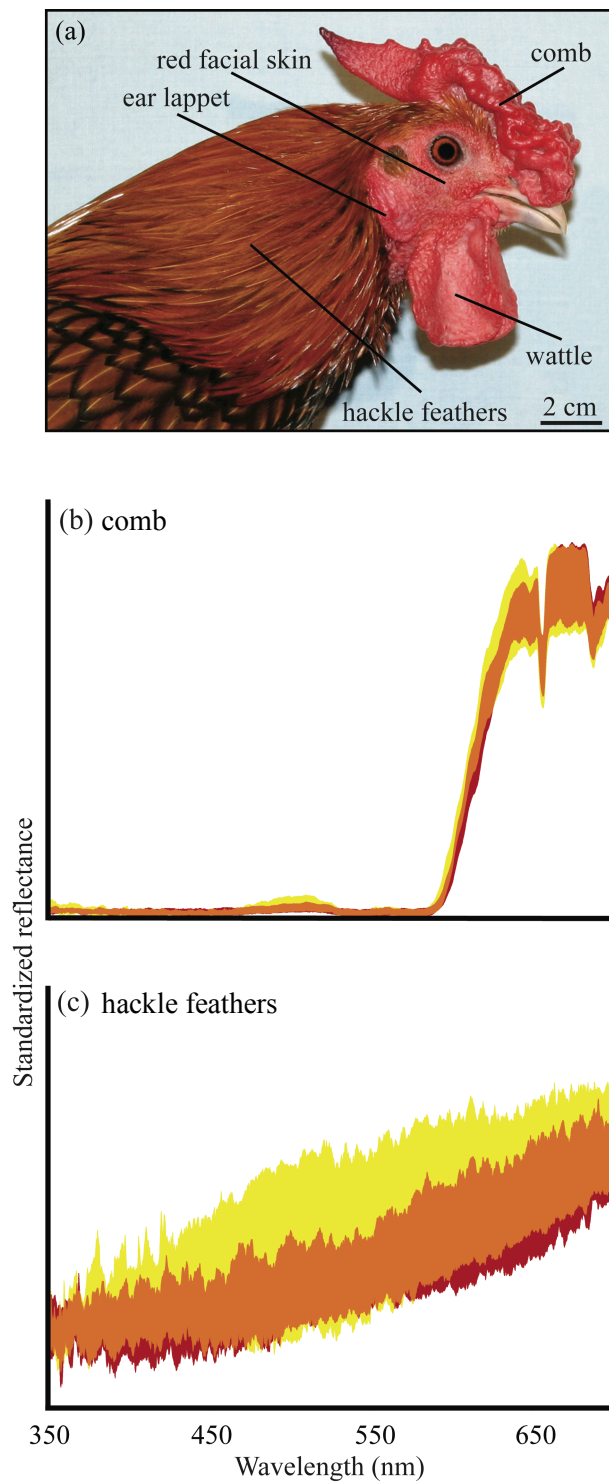


Figure 1. Feather and ornament colour do not predict male mating success. Shown (a) are the hackle feathers and four fleshy red ornaments, including the comb, wattle, ear lappet, and red facial skin. Standardized reflectance of the comb (b) and hackle feathers (c) is presented on the ordinate (0-1) and wavelength on the abscissa (350-700 nm). For each character (comb and hackle feathers), interquartile

ranges are plotted for the 10 males from season 3 with the lowest mating success (yellow) and the 10 males with the highest mating success (red). The interquartile ranges of the two groups overlap (orange) considerably across the full range visible to females.

Figure 2.

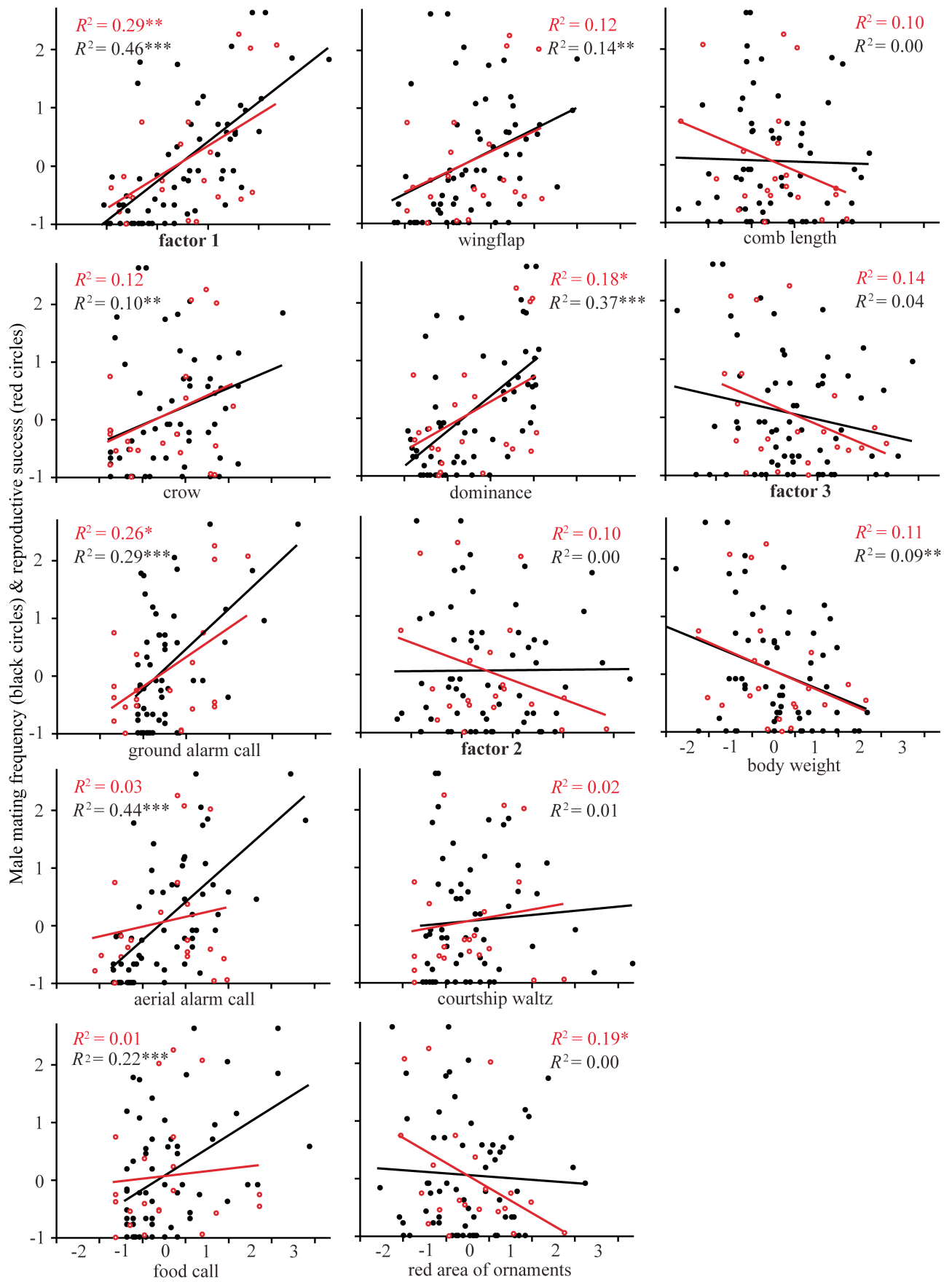


Figure 2. Behaviour predicts both mating frequency (black filled circles, $N = 64$) and reproductive success (open red circles, $N = 21$) in naturalistic social groups. Abscissa represent three orthogonal principal components and ten original variables. Each factor is followed immediately by the variables(s) from which it was derived. Factor loading scores are presented in Table 3. Data are expressed as standard deviates to facilitate comparisons across variables. R -squared values, statistical significance (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.0001$), and trendlines fitted using the least squares method are presented on each graph. The slopes of the regression lines for mating and reproductive success differ statistically only for aerial alarm calls (t -test: $t = 2.32$, $P < 0.05$; all other $P > 0.1$).